Stable production cell lines that secrete high quantities of bikunin were developed by transfecting CHO(dhfr-) cells with the expression vector shown in Figure 1. The vector was constructed using standard recombinant DNA techniques as described in U.S. Pat. No. 5,612,213 to Chan and in Sambrook et al., 1989 (supra). The expression vector contains discrete expression cassettes for the bikunin gene (truncated bikunin – amino acid sequence given in Figure 2) and the amplifiable and selectable gene DHFR (dihydrofolate reductase). About 1 x 10^6 CHO (Chinese hamster ovary) cells were transfected with 10 μg of pBC-BK using Lipofectin reagents (Life Technology, Bethesda, Maryland) according to manufacturer's instructions. The cells were then selected in the presence of 50 nM methotrexate and grown in DME/F12 media deficient in thymidine and hypoxanthine plus 5% dialyzed fetal bovine serum. Cell populations were screened for bikunin production with a chromogenic assay. Briefly, bikunin standards or culture fluid was serially diluted and incubated with an equal volume of kallikrein at 37° C for 30 minutes after which a chromogenic substrate, N-benzoyl-Pro-Phe-Arg-pNA, was added. The reaction was incubated for 15 minutes before the addition of 50% acetic acid. The amount of p-nitroanilide released was measured at 405 nM. The high producing populations were further selected in media containing increasing concentrations of methotrexate (100 to 400 nM methotrexate) and screened for the production of bikunin. Limiting dilution cloning was then applied to derive clones with high and stable productivity. The cloning was done in the absence of methotrexate using standard tissue culture techniques by depositing 1 cell/well in 96-well plates. A clone designated FD3-1 was chosen for productivity evaluation in a bioreactor and was deposited on November 12, 1999 with the American Type Culture Collection (ATCC), Rockville, MD, and was assigned accession number PTA-940.

In the Claims

Please cancel claims 3-5 and 16-17.

Please amend claims 2 and 15 as follows.

2. (Amended) An isolated mammalian glycosylated bikunin, wherein the glycosylated bikunin comprises at least one sialic acid residue.